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Abstract

Background: Dichlorodiphenyltrichloroethane (DDT) was used extensively to control malaria, typhus, body lice, and bubonic plague worldwide, until countries began restricting its use in the 1970s. However, use of DDT to control vector-borne diseases continues in developing countries. Prenatal DDT exposure is associated with elevated blood pressure in humans.

Objective: We hypothesized that perinatal DDT exposure caused hypertension in adult mice.

Methods: DDT was administered to C57BL/6J dams from gestational day 11.5 to postnatal day 5. Blood pressure (BP) and myocardial wall thickness were measured in male and female adult offspring. Adult mice were treated with an angiotensin converting enzyme (ACE) inhibitor, captopril, to evaluate sensitivity to amelioration of DDT-associated hypertension by ACE inhibition. We further assessed the influence of DDT exposure on the expression of mRNAs that regulate BP through renal ion transport.

Results: Adult mice perinatally exposed to DDT exhibited chronically increased systolic BP, increased myocardial wall thickness, and elevated expression of mRNAs of several renal ion transporters. Captopril completely reversed hypertension in mice perinatally exposed to DDT.

Conclusions: These data demonstrate that perinatal exposure to DDT causes hypertension and cardiac hypertrophy in adult offspring. A key mechanism underpinning this hypertension, is an overactivated renin angiotensin system, since ACE inhibition reverses the hypertension induced by perinatal DDT exposure.

Introduction

Cardiovascular disease is the number one cause of overall mortality throughout the world (Yusuf et al. 2015) for which hypertension is a major risk factor (Wilkins et al. 2012). Hypertension afflicts over a quarter of the world's adults, with nearly twice as many cases of hypertension in the developing world compared to the developed world (Kearney et al. 2005).

Most cases of hypertension are considered essential hypertension for which a clear cause is not clinically identifiable (Messerli et al. 2007). It may be postulated that some of these cases of hypertension arise from causes such as perturbations in fetal or early life development. This postulate is supported by strong epidemiological and experimental evidence. For example, fetal malnutrition results in hypertension and cardiovascular disease in adulthood (Alwasel and Ashton 2009; Barker et al. 1989; Barker et al. 1993; Woods et al. 2001). Furthermore, perinatal administration of the glucocorticoid dexamethasone causes hypertension and an increase in the expression of renal ion transporters in adult rat offsprings (Dagan et al. 2008). These observations support the paradigm that the nutritional environment of the fetus during critical developmental periods may lead to impaired blood pressure control in adulthood.

There is some evidence that perinatal exposure to environmental toxicants can cause cardiovascular disease in adulthood. For example, prenatal exposure to the pesticide dichlorodiphenyltrichloroethane (DDT) is associated with increased medicated hypertension in adult women (La Merrill et al. 2013), and prenatal exposure to its metabolite dichlorodiphenyldichloroethylene (DDE) is associated with elevated blood pressure in four year old children (Vafeiadi et al. 2015). However, whether DDT or DDE burden in *adults* is associated with hypertension remains somewhat controversial since some studies do (Henriquez-Hernandez et al. 2014; Lind et al. 2014; Siddiqui et al. 2002) and some studies do

not report an association DDT burden and hypertension in adult offspring (Goncharov et al. 2011; Savitz et al. 2014; Valera et al. 2013).

This led us to hypothesize that perinatal exposure to DDT is a key risk factor for hypertension in the adult. The present study seeks to experimentally test the hypothesis that developmental exposure to DDT causes hypertension in adult offspring of mice.

Given the continual use of DDT and the presence of its metabolite DDE in particular in developing nations, even a modest effect of DDT or its metabolites on blood pressure or cardiovascular disease may have far-reaching public health implications.

Methods

Drugs.

p,p'-DDT (98.5% purity neat) and o,p'-DDT (100% purity neat) were purchased from AccuStandard (New Haven, CT). To simulate the relative abundance of these congeners in the commercial formulation of DDT used as a pesticide in the US prior to its ban, 77.2% p,p'-DDT and 22.8% o,p'-DDT were dissolved in organic olive oil at a final concentration of 0.17 g DDT mixture/L (Ecobichon and Saschenbrecker 1968), hereafter referred to as DDT. Captopril (98% purity) was purchased from Sigma-Aldrich (St. Louis, MO). Captopril was dissolved at a concentration of 0.49 mg/ml and 0.57 mg/ml in drinking water of female and male mice, respectively, based on the water intake of 2 co-housed male or female mice that had prior exposure to the DDT dose used here or its control (see Supplemental Material, Table S1).

Mice. Virgin 8 week old C57BL/6J male and female mice were ordered from Jackson Laboratories and acclimated for 1-2 weeks prior to timed mating.

DDT exposure and mouse husbandry. We administered 1.7 mg DDT/kg body weight (p. o., n = 15 dams, 10 μL solution/kg mouse), or the equivalent volume of olive oil vehicle (hereafter referred to as vehicle, p. o., n = 14 dams) daily to primigravid C57BL/6J dams from 11.5 days post coitus (DPC) to postnatal day (PND) 5 to span a developmental exposure window important for rodent kidney and heart function (Aragon et al. 2008; Couture et al. 1990; Lin et al. 2001; Thackaberry et al. 2005; Xu et al. 2011). We previously reported mean (± SEM) maternal serum levels of (in ng/ml serum) 2.2 (± 0.1) p,p'-DDE, 51.1 (± 10.2) p,p'-DDT, and did not detect o,p'-DDT on PND 6, 24 hrs after an identical daily dosing protocol of 1.7 mg/kg from 11.5 DPC to PND5 (La Merrill et al. 2014). These exposures are within the range of past and contemporary human serum levels of both p,p'-DDT and p,p'-DDE (Bouwman et al. 1992; Cox et al. 2007; Gauthier et al. 2014; Herrera-Portugal et al. 2005; La Merrill et al. 2013; Rylander et al. 2009; Vafeiadi et al. 2015).

After the final DDT dose on PND 5, we culled litters to 6 pups to equalize litter size. At PND 21, we weaned pups (1 cage/sex/litter/treatment) for later experiments. All mice had access to food and water *ad libitum* in sterile ventilated cages in an American Association for the Accreditation of Laboratory Animal Care-approved facility on a 12/12 light cycle corresponding to 700/1900 h. At the end of the studies, mice were sacrificed by exsanguination under isoflurane anesthesia. All procedures were in accordance with the UC Davis and Mount Sinai School of Medicine IACUC protocols.

Blood pressure measurements and angiotensin converting enzyme (ACE) inhibitor treatment.

Based on our previous finding that prenatal DDT exposure increased risk of hypertension in adult women (La Merrill et al. 2013), we assessed the development of adult male and female mouse hypertension (Monassier et al. 2006) by measuring blood pressure with a volume pressure recording sensor and an occlusion cuff (CODA, Kent Scientific) on the tail. We recorded 18 volume pressure cycles per restrained mouse in 2 mice/sex/litter/perinatal treatment group when they were 5 months old after a day of acclimation to the procedure (see Supplemental Material, Table S2).

We next tested whether an over-activated renin angiotensin system (RAS) could be a key contributor of DDT effects by treating 7 month old (adult) male and female mice with an angiotensin converting enzyme inhibitor (ACEi) and measuring blood pressure by two independent methods in the ACEi studies (see Supplemental Material, Table S2).

We recorded 18 volume pressure cycles per tail cuffed- and restrained- mouse in 2 male and 2 female mice for 7 litters/perinatal treatment group prior to the initiation of captopril treatment ("ACEi") or untreated water ("WATER"). This was repeated both 6 and 7 days after the initiation of 7 days of ACEi or WATER to assure sufficient time for pharmacological efficacy (Emanueli et al. 1997). Mice were accustomed to the tail cuff procedure for a day prior to the measurements. Daily water intake was quantified before captopril treatment to appropriately target a daily intake of approximately 120 mg captopril/kg body weight (Emanueli et al. 1997). Daily water intake during 7 days of daily captopril treatment indicated an average daily dose of 120 and 109 mg captopril/kg in female and male mice, respectively (see Supplemental Material, Table S1).

To validate these non-invasive blood pressure measurements conducted on restrained mice, we assessed blood pressure through an indwelling telemetric blood pressure device in a subgroup of unrestrained male mice (see Supplemental Material, Table S2; PA-C10, DSI, St. Paul, MI). Transmitters were implanted into the aortic arch of 6 male mice/perinatal treatment during isoflurane anesthesia. Mice recovered from surgery for 5 days, receiving analgesic buprenorphine twice daily for 3 days following surgery. Blood pressure was assessed in 7 month old male mice by telemetry for 1 hour daily from 1700-1800 h for 5 days ("WATER") and from days 7-12 ("ACEi"). We added captopril to the drinking water on the fifth day (see Supplemental Material, Table S1). Due to some postsurgical mortality and device malfunctions, not all males that underwent surgery completed the ACEi study, resulting in 6 males in the VEHICLE+WATER, 4 males in the DDT+WATER, 3 males in the VEHICLE+ACEi and 4 males in the DDT+ACEi treatment groups.

While 7 month old male mice were subjected to telemetric blood pressure measurements, their age-matched sisters were subjected to an additional volume pressure recording of 18 volume pressure cycles per tail cuffed- and restrained- mouse (1 female mouse/litter and 6 litters/treatment; see Supplemental Material, Table S2) prior to euthanasia. This was to confirm that the perinatal treatment effect was present at the time of transcript analysis (see section "semi-quantitative PCR" below).

Cardiac echography. Because we hypothesized that subtle increases in blood pressure resulting from perinatal DDT exposure could lead to cardiac hypertrophy, we evaluated cardiac phenotypes of male and female mice by echocardiography a month after significantly elevated blood pressure was observed in both sexes (see Supplemental Material, Table S2). Echocardiography was performed on 8 month old mice (1 mouse/sex/litter, 7

litters/treatment, 14 mice/treatment) under sedation by intraperitoneal ketamine up to 75 mg/kg. Sedation was optimized by giving the lowest dose of ketamine needed to (1) restrain the animal and prevent motion artifact (2) maintain the heart rate in the range of 550-650 beats/minute. Ketamine was chosen based on our previous experience and considering that alternative agents had either a long duration of action (pentobarbital), were potentially unsafe due to increased cardiac toxicity, or might cause a bradycardic effect (isoflurane, ketamine/xylazine) as demonstrated elsewhere (Stein et al. 2007). Short-axis parasternal views of the left ventricle (LV) at the mid-papillary level were obtained using a Vivid i echocardiography apparatus with a 13MHz linear array probe (General Electric, New York, NY) on mice with hair removed (Nair). M-mode measurements of the size of the LV walls and cavities were obtained by 2D guidance from the short-axis view of the LV as recommended by the American Society of Echocardiography (Lang et al. 2005). Three different measurements in diastole (d) were averaged per animal to estimate LV wall thicknesses (septum and posterior wall) and LV dilation (internal diameter).

Pathology. After 8 month old mice had completed echography, kidneys were harvested from 1 mouse/sex/perinatal treatment and subjected to routine hematoxylin and eosin processing (see Supplemental Material, Table S2). Histopathological evaluations were assessed by a veterinary pathologist who was blinded to the treatments.

Semi-quantitative PCR. Renal mRNA was extracted (Qiagen, Hilden, Germany) from 7 month old female mice to synthesize cDNA using reverse transcription PCR (Applied Biosystems, Foster City, CA). Semi-quantitative PCR was performed using SybrGreen

probes (Applied Biosystems) with primers for transcripts: sodium hydrogen exchanger 1 (*Slc9a1*, F: CCTGACCTGGTTCATCAACA, R: TCATGCCCTGCACAAAGACG (Stiernet et al. 2007)); sodium hydrogen exchanger 2 (*Slc9a2*, F: TGGCAGAGACAGGGATGATAAG, R: CCGCTGACGGATTTGATAGAGATTC (Stiernet et al. 2007)); sodium hydrogen exchanger 3 (*Slc9a3*, F: GCACACAACTACACCATCAAGG, R: AGGGGAGAACACGGGATTATC (Stiernet et al. 2007)); sodium hydrogen exchanger 4 (*Slc9a4*, F: CGGAGGAACCTGCCAAAATC, R: CGGAGGAACCTGCCAAAATC (Stiernet et al. 2007)); sodium phosphate transporter (*Slc34a1*, F: GCTGTCCTCTACCTGCTCGTGTG, R: GCGTGCCCACTCCGACCATAG (Marsell et al. 2008)); sodium potassium ATPase subunit 1 (*Atp1a1*, F: CGGAATTCATGCGGAGGATGTCGTC, R: GCCGCTCGAGGTGGATGAAATGCTCAAT (Klesert et al. 2000)); sodium potassium ATPase subunit 2 (*Atp1a2*, F: GAATGGGTTTCTACCATCGCG, R: GCACAGAACCACCACGTGAC (Marsell et al. 2008)); sodium calcium exchanger 1 (*Slc8a1,* F: TGAGAGGGACCAAGATGATGAGGAA, R: TGACCCAAGACAAGCAATTGAAGAA (Otsu et al. 2005)); sodium potassium chloride cotransporter (Slc12a2, F: GAACCTTTTGAGGATGGC, R: CACGATCCATGACAATCT (Castrop et al. 2005)); sodium potassium chloride cotransporter (*Slc12a1*, F: TGCTAATGGAGATGGGATGC, R: CAGGAGAGGGCAATGAAGAG (Alshahrani et al. 2012)). The 2^{-ddCT} method (Livak and Schmittgen 2001) was used with 18s (*s18,* F: TTGACGGAAGGGCACCACCAG, R: GCACCACCACCCACGGAATCG (Au et al. 2011)) as an endogenous control in kidneys to calculate transcript fold change.

Statistical analyses. The normal distribution was evaluated for all outcome variables here. Tail blood pressure was assessed by modeling the fixed effect of perinatal DDT and the random effect of litter (PROC MIXED, SAS, Raleigh, NC). In the ACEi study, ACEi and an ACEi*perinatal treatment term were modeled as fixed effects in the tail blood pressure model. Models of arterial blood pressure measured by telemetry included the random effect of litter using PROC GLIMMIX, a model which does not require a normal outcome distribution. mRNA expression was evaluated without random effects because only one mouse per litter was analyzed (PROC GLM, SAS). We stratified by sex in all outcomes for which both sexes were evaluated.

Results

Perinatal DDT elevates blood pressure in adult offspring. To assess whether the association between prenatal DDT exposure and blood pressure in adults is causal (La Merrill et al. 2013), we exposed fetuses and nursing pups to DDT by gavaging dams perinatally and measuring the blood pressure of adult offspring at 5- and 7- months old. Blood pressure was assessed through two methods, non invasively through tail cuff blood pressure monitoring of restrained mice (Figures 1-3) and invasively through telemetric blood pressure monitoring (Figure 2E-F).

Male offspring perinatally-exposed to DDT had elevated systolic and diastolic blood pressure when five months old (Figure 1A). A similar but statistically non-significant trend was seen in five month old female offspring (Figure 1B). By 7 months of age, both male and

female offspring exposed perinatally to DDT had increased systolic blood pressure ($p<0.05$, Figure 2A, 2C, and 3A).

To evaluate whether perinatal DDT increased blood pressure by increasing the activity of the RAS (Woods et al. 2001), we administered the ACE inhibitor captopril for 5-7 days to adult offsprings in the study. Indeed, ACE inhibition lowered blood pressure in 7 month old mice exposed to DDT to a greater extent than observed in the vehicle group (Figure 2; see Supplemental Material, Table S3) demonstrating that a primary cause of hypertension (Monassier et al. 2006) induced by perinatal DDT exposure is an over-activated RAS.

The well known role of aldosterone in inducing sodium transport to elevate blood pressure (Rozansky 2006) led us to hypothesize that an over-activated RAS led to increased blood pressure via increased sodium transporter transcription in DDT exposed mice. We tested this by evaluating expression of sodium transporter mRNA in female mice when they were 7 months old, the earliest time point at which we could detect a significant elevation in the blood pressure of female mice perinatally exposed to DDT (Figure 3A). Indeed, perinatal DDT exposure induced the mRNA expression of several Na+/H+ exchangers, two Na+/K+/Cl- cotransporters, a subunit of the Na+/K+ ATPase, and a Na+/Ca2+ exchanger in hypertensive kidneys (Figure 3B), consistent with elevated expression of renal ion transporters in hypertension caused by an over-activated RAS (Rozansky 2006).

To rule out that DDT causes hypertension by impairing nephrogenesis (Langley-Evans et al. 1999), kidneys collected from 8 month old male and female mice were examined by a veterinary pathologist. Histological assessment of the kidneys was completely normal at the light microscopy level (see Supplemental Material, Figure S1), supporting the notion that the cause of the hypertension induced by DDT is not structural in nature.

Perinatal DDT increases heart wall thickness in adult offspring.

Chronic hypertension can induce left ventricular hypertrophy due to pressure overload (Krumholz et al. 1993). We used echocardiography to investigate this possibility in 8 month old male and female mice (a month after the last blood pressure measurements). We found that perinatal DDT exposure led to a significant increase in left ventricular wall thickness of both posterior wall and septum in female mice (Figure 4A-B), as to be expected with chronic pressure overload. This data is consistent with the observation that hypertension in women favors the development of concentric hypertrophy (Krumholz et al. 1993).

Despite the observation that male mice exposed to DDT had an earlier onset of hypertension, we did not observe any difference in ventricular wall thickness (Figure 4D-F). Further, there was no evidence of volume overload as assessed by left ventricular dilation (Figure 4C and 4F).

Discussion

Here we investigated the effects of perinatal DDT exposure on cardiovascular health in adult offsprings. To our knowledge, this is the first study to establish that perinatal DDT exposure is sufficient to cause hypertension and cardiac hypertrophy in adult offspring (Monassier et al. 2006). Remarkably, the increase in blood pressure caused by perinatal DDT was normalized by treatment with an ACE inhibitor, demonstrating that an over-activated RAS is a key mechanism in perinatal DDT exposure induced hypertension. In turn, over activated RAS was associated with increased renal expression of sodium channels.

The maternal serum levels of DDT and DDE that were achieved in this study were similar to the range of DDT and DDE levels observed in human maternal serum levels;

particularly to those that were associated with high blood pressure during childhood and hypertension in adult offspring (La Merrill et al. 2013; Vafeiadi et al. 2015). Thus, perinatal DDT exposure could be an important contributor in some of the hypertension that is currently still classified as "essential". Our study mechanistically underpins associative evidence in humans that the latent effects of perinatal DDT exposure can clinically manifest (La Merrill et al. 2013) decades after environmental and human exposure levels have dramatically declined (Ritter et al. 2009). This research therefore provides a salient example for the legacy of detrimental health effects from persistent organic pollutants.

Interestingly, captopril caused a greater reduction of blood pressure in mice perinatally exposed to DDT than in controls, consistent with an over-activated RAS as a primary cause of DDT induced hypertension. An over-activated RAS may also explain the increased expression of several ion transporters in the DDT-exposed hypertensive adult offspring since the expression of these transporters is regulated by the RAS (Rozansky 2006). Future studies should evaluate the effect of ACE inhibition on altered renal sodium channel expression after perinatal DDT exposure to confirm that DDT effects on sodium channel expression are RAS mediated.

We observed modest concentric hypertrophy in the hearts of female adult mice perinatally exposed to DDT, likely due to the hypertension (Monassier et al. 2006). This sexual dimorphism of greater cardiac wall thickness of hypertensive females has also been observed in humans (with some contention), where hypertensive women are more likely to develop concentric (Krumholz et al. 1993) and severe (Topol et al. 1985) cardiac hypertrophy than hypertensive men. Given that cardiac hypertrophy is associated with a higher risk of cardiovascular mortality in women than in men (Levy et al. 1990), future studies will need to elucidate the mechanism of the sex-specific results here, as inherent sex-differences in

cardiac hypertrophy could account for some of the sexual dimorphism of hypertrophy in DDT exposed mice (Krumholz et al. 1993; Topol et al. 1985). Further whether developmental DDT exposure is associated with cardiac hypertrophy in humans, and if so whether this is purely due to the hypertension is a critical outstanding question which will need to be addressed.

The strengths of this study are the measurements of blood pressure by two independent methods with similar results. Each of these methods has particularly strength, such as no need for surgery and or lack of restraint stress and hence reaffirms the independent measurements. The study is further strengthened by the assessment of cardiac hypertrophy by echocardiography. Our dosing paradigm results in perinatal maternal levels of DDT and DDE that fall within the range of the top tertile based on the only human study that has evaluated and found a positive association between prenatal DDT and adult hypertension, which adds to the translational value of the study (La Merrill et al. 2013). A limitation of the study is that we do not have a control group where we induced a similar DDT burden in adulthood and assessed the effects on hypertension since we cannot rule out that the hypertension in adult offspring is due to the remaining DDT burden associated with to its long halflife. The increase in blood pressure with age while DDT burden decreases over time, suggests that any DDT remaining in our mice at the time of blood pressure assessment was not a major driver of the hypertension we observed. Finally, whether ACE inhibition reduces transcripts associated with renal ion transport that we found elevated by perinatal DDT exposure remains to be determined.

Conclusions.

Our data demonstrate that perinatal DDT exposure causes hypertension in both female and male adult mice due to an over-activation of the RAS. This chronic over-activation of the

RAS was associated with increased renal expression of sodium channels. Further, perinatal DDT caused cardiac hypertrophy, possibly through pressure overload. These observations support the hypothesis that perinatal exposure to DDT is a risk factor for hypertension and cardiovascular disease in adult offsprings.

On a positive note, our study provides evidence that ACE inhibition normalizes hypertension of mice perinatally exposed to DDT. ACE inhibitors are among the most commonly used antihypertensive drugs that have been demonstrated to be safe and to reduce mortality from heart disease (Li et al. 2014), indicating that they would be the drug of choice for the treatment of hypertensive individuals with high early-life DDT exposure. Given that the ban of DDT in the US has reduced DDT exposure among the US population (Ritter et al. 2009), ongoing replacement of DDT with other malaria vector controls is likely to further decrease worldwide DDT exposures over time. Our study suggests this may reduce the susceptibility to cardiovascular disease of future generations.

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Figure Legends

Figure 1. Perinatal DDT exposure increases blood pressure of 5 month old male mice. Systolic and diastolic blood pressure (tail cuff) in five month old (A) male and (B) female mice (DDT p=0.1). N = 2 mice/sex/litter, 15 DDT litters and 14 vehicle-exposed litters.

*p<0.05, DDT vs. vehicle controls.

Figure 2. Increased blood pressure by perinatal DDT exposure is reduced by ACE inhibition in 7 month old male and female mice. (A) Systolic- (DDT*ACEi pi=0.02, N = 2 mice/litter, 7 litters/treatment) and (B) diastolic- tail blood pressure (DDT*ACEi pi=0.002, N = 2 mouse/litter, 7 litters/treatment) in female mice, and (C) systolic- (DDT*ACEi pi=0.05, N = 2 mouse/litter, 7 litters/treatment) and (D) diastolic- (DDT*ACEi pi=0.19, N = 2 mouse/litter, 7 litters/treatment) tail blood pressure in male mice at 7 months old immediately prior to- and after- one week of captopril exposure in drinking water. (E) Systolic- (DDT*ACEi pi<0.0001) and (F) diastolic- (DDT*ACEi pi<0.0001) aortic blood pressure in 7 month old male mice (N = 6, 4, 3, and 4 for VEH- and DDT- treated at baseline and VEH- and DDT- treated during captopril, respectively).

*p<0.05, **p<0.01, *** p<0.0001.

Figure 3. Perinatal DDT increases blood pressure and renal sodium transporter expression in 7 month old female mice. (A) Systolic- and diastolic- blood pressure immediately prior to the assessment of (B) renal sodium transporter transcripts in 7 month old

female mice that were not subjected to the ACE inhibition experiment. N = 1 female mouse/litter and 6 litters/treatment.

*p<0.05, **p<0.01, ***p<0.0001 DDT vs. vehicle controls.

Figure 4. Perinatal DDT causes cardiac hypertrophy in 8.5 month old female mice. (A) Left ventricular posterior wall thickness-, (B) left ventricular septum thickness-, and (C) left ventricular dilation- in diastole in 8 month old female mice. (D) Left ventricular posterior wall thickness-, (E) Left ventricular septum thickness-, and (F) left ventricular dilation- in diastole of male mice. N = 1 mouse/sex/litter, 7 litters/treatment.

**p<0.01 DDT vs. vehicle controls.

Figure 1.

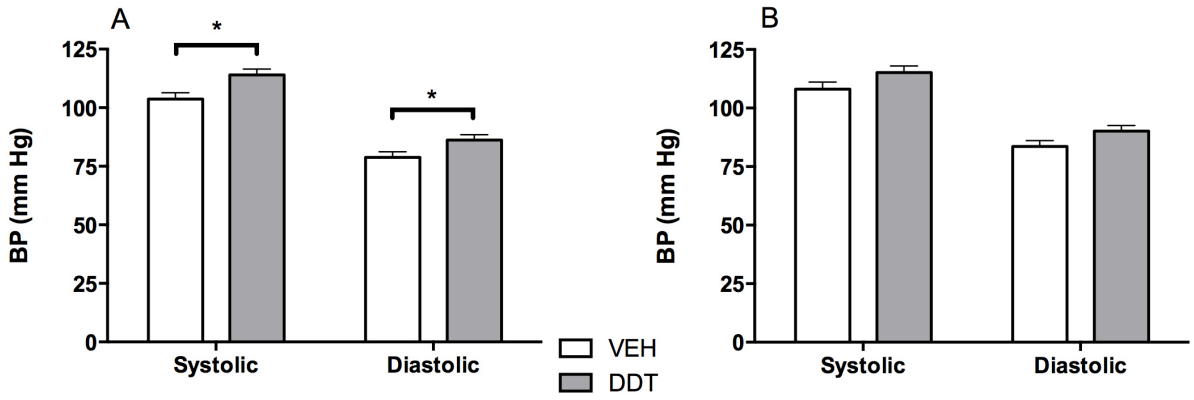


Figure 2.

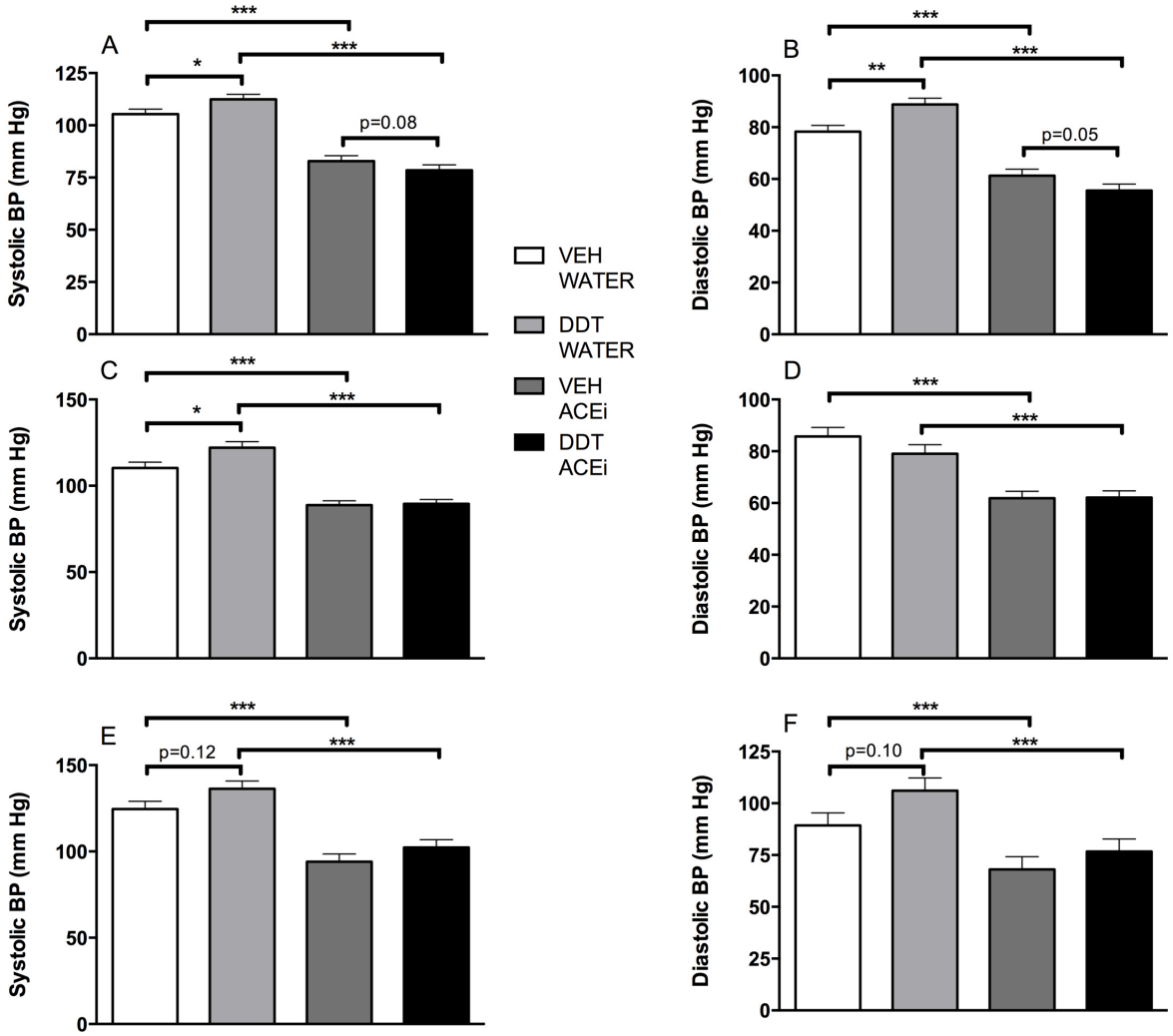

A
B
C
D
E
F
Systolic BP (mm Hg)
Diastolic BP (mm Hg)
p=0.08
p=0.05
p=0.12
p=0.10
*
**
***
VEH WATER
DDT WATER
VEH ACEi
DDT ACEi

Figure 3.

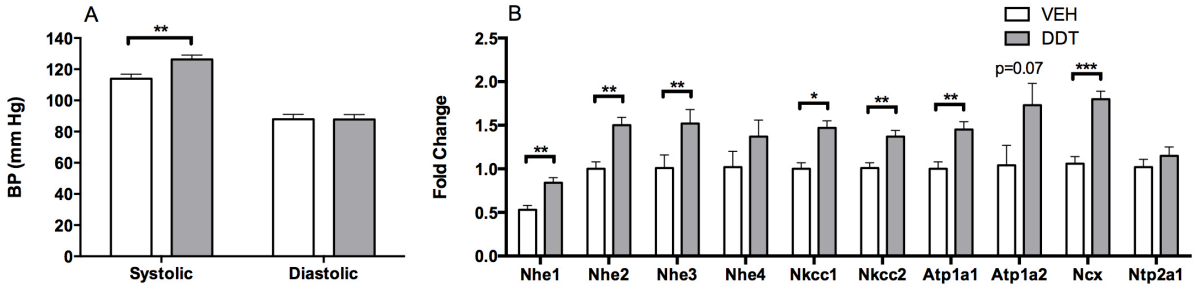
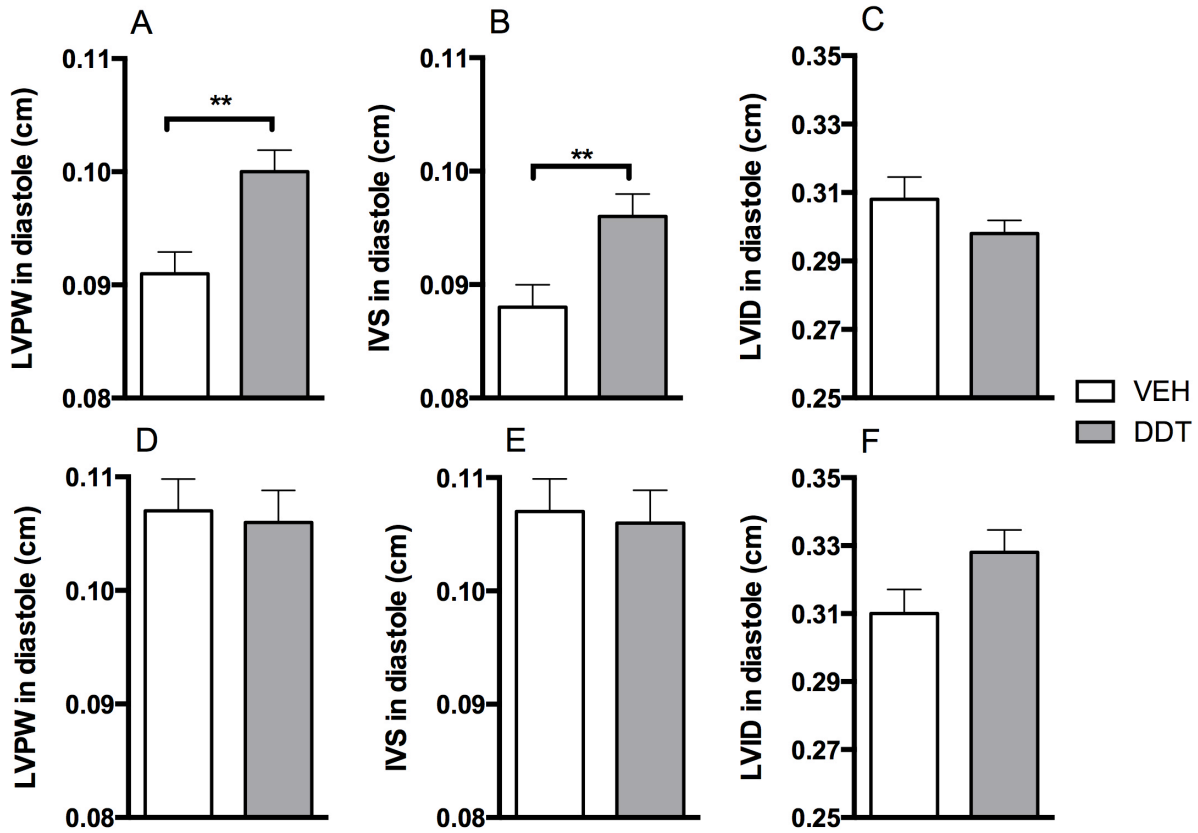

A
BP (mm Hg)
140
120
100
80
60
40
20
0
**
Systolic
Diastolic
B
Fold Change
2.5
2.0
1.5
1.0
0.5
0.0
VEH
DDT
p=0.07
**
**
**
*
**
**
***
Nhe1
Nhe2
Nhe3
Nhe4
Nkcc1
Nkcc2
Atp1a1
Atp1a2
Ncx
Ntp2a1

Figure 4.


A
B
C
D
E
F
LVPW in diastole (cm)
IVS in diastole (cm)
LVID in diastole (cm)
0.11
0.10
0.09
0.08
0.35
0.33
0.31
0.29
0.27
0.25
**
VEH
DDT